REMARKS

Claims 1-7, 17, 19-21, and 28-31 are pending. Claims 28-29 are withdrawn.

CLAIM REJECTIONS UNDER 35 U.S.C. §112

Claims 1-7, 17, 19-21 and 30-31 are rejected under 35 U.S.C. §112 ¶2 as indefinite.

Regarding the claim 1 rejection for reciting an "effective concentration", Applicants disagree at least because one of ordinary skill in the art would know an effective concentration based on the disclosure from p. 15 line 21 to p. 16 line 9. This disclosure describes exemplary and typical concentration ranges (about 0.1 mg/kg body weight to about 500 mg/kg body weight, typically about 0.5 mg/kg body weight to about 2 mg/kg body weight), conditions that may influence concentration (the particular complex employed, the organs/tissues to be examined, the particular equipment employed), how the route of administration may effect concentration (e.g., a concentration for parenteral administration ranges from about 1 μM to about 0.5 M, preferably from about 1 mM to about 10 mM), and other embodiments. One of ordinary skill in the art would also appreciate from Applicants' disclosure that effective concentrations are those that "achieve the desired diagnostic or therapeutic objective", the determination of which is routine experimentation. Further, one of ordinary skill in the art would know that patient specific factors such as gender, weight, body mass, etc. are criteria that may also pay a role in determining an effective concentration, the determination of which is routine experimentation.

Claim 1 is amended to recite "conjugate of step (f) as required.

Claim 17 is amended to recite "at least one pharmaceutical carrier or excipient" as required. The amendment is supported at least at p. 16 lines 13-25; no new matter is introduced. Claim 17 is also amended to address the Examiner's objection; this Amendment is solely to facilitate prosecution because Applicants disagree that claim 17 requires correction.

Claims 1-7 and 30 are rejected as indefinite. Applicants have amended claim 1 to conform the language of step (e) with the language of step (c), because both steps (e) and (c) recite isolating the antibodies prepared in the preceding step. One of ordinary skill in the art knows how to isolate such antibodies, e.g., using competitive binding assays where the internal image antibody would, in vitro, inhibit binding of the original antigen to the target ST receptor. Applicants assert that the claims are thus sufficiently definite.

For at least these reasons, Applicants assert that the rejections under §112 ¶2 are overcome and respectfully request their withdrawal.

Claims 2 and 7 are rejected under 35 U.S.C. §112 ¶1 as not enabled. Applicants respectfully disagree.

Regarding claim 2, it is a ligand that binds to a heat-stable toxin biological receptor; claim 2 further limits this ligand to drugs, hormones, peptides, carbohydrates, peptidomimetics, or glycomimetics. Any ligand that binds meets the claim limitation; by definition a ligand is not limited to a particular category (Ligand: when a protein molecule binds to another molecule, the second molecule is commonly referred to as a ligand. Molecular Biology of the Cell, p. 122 (attached); Ligand: A linking or binding molecule. Immunology Fifth Edition p. 408 (attached)). A ligand may thus be a drug, hormone, peptide, carbohydrate, peptidomimetic, or glycomimetic, as Applicants' claim recites.

Regarding claim 7, it is in a patient target site containing the heat-stable toxin biological receptor at which the conjugate accumulates; claim 7 further limits this target site to a tumor, lesion, necrotic region, ischemic region, thrombic region, inflammatory region, and/or impaired vasculature that contains the heat-stable toxin biological receptor. Any target site containing the heat stable toxin receptor meets the claim limitation, whether it be a lesion (a general term known to one of ordinary skill in the art), and whether the tissue be necrotic, ischemic, thrombic, inflammatory, or have impaired vasculature. Thus Applicants assert that claims 2 and 7 are sufficiently enabled.

Claims 1-7 and 30 are rejected under 35 U.S.C. §112 ¶1 as not enabled. Applicants have amended the claims as the Examiner has suggested to overcome this rejection.

For at least these reasons, Applicants assert that the rejections under 35 U.S.C. §112 are overcome and request their withdrawal.

CONCLUSION

Applicants believe no fees are due but, if deemed necessary, such fees are authorized to be charged to Deposit Account No. 20-0809.

The Examiner is invited to contact Applicants' undersigned representative with questions.

Respectfully submitted,

THOMPSON HINE, LLP

/Beverly A. Lyman/
Beverly A. Lyman, Ph.D.
Reg. No. 41,961

312 Walnut Street
14th Floor
Cincinnati OH 45202
513 352 6596
513 241 4771 facsimile

657700

IMMUNOLOGY

FIFTH EDITION

IVAN ROITT
MA DSc (Oxon) Hon FRCP (Lond) FRCPath FRS

Emeritus Professor of Immunology
Director of Institute of Biomedical Science
University College London Medical School
London, UK

JONATHAN BROSTOFF
MA DM (Oxon) DSc FRCP (Lond) FRCPath

Professor of Allergy and Environmental Health
Centre for Allergy Research
Department of Immunology
University College London Medical School
London, UK

DAVID MALE
MA PhD

Senior Lecturer in Neuroimmunology
Department of Neuroimmunology
Institute of Psychiatry
London, UK

Mosby
London Philadelphia St Louis Sydney Tokyo

Publisher	**Dianne Zack**
Managing Editor	**Louise Crowe**
Project Manager	**Cathryn Waters**
Design	**Pete Wilder**
Layout	**Rob Curran**
Cover Design	**Greg Smith**
Illustration Manager	**Lynda Rosemary Payne**
Illustrators	**Richie Prime**
	Sandie Hill
	Lynda Rosemary Payne
Production	**Gudrun Hughes**
Index	**Liza Weinkove**

Published by Mosby, an imprint of Mosby International Limited, Lynton House, 7–12 Tavistock Square, London WC1H 9LB, UK

ISBN 0 7234 2918 9

First published by Gower Medical Publishing Ltd, 1985, ISBN 0-906923-35-2

Second Edition published by Gower Medical Publishing Ltd, 1989, ISBN 0-3974-4573-3

Third Edition published by Mosby–Year Book Europe Ltd, 1993, ISBN 0-397-44765-5

Fourth Edition published by Mosby, an imprint of Times Mirror International Publishers Ltd, 1996, ISBN 0-7234-2178-1

Printed by Butler & Tanner, Somerset, UK

Text set in Galliard; captions set in Univers 55

For full details of all Mosby titles, please write to Mosby International Publishers Limited, Lynton House, 7–12 Tavistock Square, London WC1H 9LB, UK.

A CIP catalogue record for this book is available from the British Library.

Library of Congress Cataloging-in-Publication Data has been applied for.

LFAs (leucocyte functional antigens). A group of three molecules which mediate intercellular adhesion between leucocytes and other cells in an antigen non-specific fashion. LFA-1 is CD11a/CD18, LFA-2 is CD2 and LFA-3 is CD58.

Ligand. A linking (or binding) molecule.

Line. A collection of cells produced by continuously growing a particular cell culture *in vitro*. Such a cell lines will usually contain a number of individual clones.

Linkage. The condition where two genes are both present in close proximity on a single chromosome and are usually inherited together.

Linkage disequilibrium. A condition where two genes are found together in a population at a greater frequency than that predicted simply by the product of their individual gene frequencies.

LPS (lipopolysaccharide). A product of some Gram-negative bacterial cell walls which can act as a B-cell mitogen.

Lymphokines. A generic term for molecules other than antibodies which are involved in signalling between cells of the immune system and are produced by lymphocytes (*cf.* interleukins).

Lymphokine activated killer cells (LAKs). Cytotoxic cells generated *ex vivo*, by stimulation with IL-2 and possibly other cytokines.

Ly antigens. A group of cell surface markers found on murine T cells which relate to the differentiation of T-cell subpopulations. Many are now assigned to the CD system.

Lytic pathway. The complement pathway effected by components C5–C9 that is responsible for lysis of sensitized cell plasma membranes.

MALT (mucosa-associated lymphoid tissue). Generic term for lymphoid tissue associated with the gastrointestinal tract, bronchial tree and other mucosa.

Mast cells. Cells found distributed near blood vessels in most tissues, that are full of granules containing inflammatory mediators.

Membrane attack complex (MAC). The assembled terminal complement components C5b–C9 of the lytic pathway; MAC becomes inserted into cell membranes.

Memory cells. Long-lived lymphocytes which have already been primed with their antigen, but have not undergone terminal differentiation into effector cells. They react more readily than naive lymphocytes when restimulated with the same antigen.

MHC (major histocompatibility complex). A set of genes found in all mammals whose products are primarily responsible for the rapid rejection of grafts between individuals, and function in signalling between lymphocytes and cells presenting antigen.

MHC restriction. A characteristic of many immune reactions in which cells cooperate most effectively with other cells with which they share an MHC haplotype.

MIF (migration inhibition factor). A group of peptides produced by lymphocytes which are capable of inhibiting macrophage migration.

Mitogens. Substances which cause cells, particularly lymphocytes, to undergo cell division.

MLR/MLC (mixed lymphocyte reaction/mixed lymphocyte culture). Assay system for T-cell recognition of allogenic cells in which response is measured by proliferation in the presence of the stimulating cells.

Mononuclear phagocyte system. The lineage of fixed and mobile long-lived phagocytic cells, related to blood monocytes and tissue macrophages.

Myeloid cells. The lineages of bone-marrow-derived phagocytes, including neutrophils, eosinophils and monocytes.

Myeloma. A lymphoma produced from cells of the B-cell lineage.

N regions. Gene segments present in recombined antigen receptor genes which are not present in the germline DNA.

Neoplasm. A synonym for cancerous tissue.

Network theory. A proposal first put forward by Jerne (since developed) which states that T cells and B cells mutually inter-regulate by recognizing idiotypes on their antigen receptors.

NK (natural killer) cells. A group of lymphocytes which have the intrinsic ability to recognize and destroy some virally infected cells and some tumour cells.

Nude mouse. A genetically athymic mouse which also carries a closely linked gene producing a defect in hair production.

Opsonization. A process by which phagocytosis is facilitated by the deposition of opsonins (e.g. antibody and C3b) on the antigen.

PAF (platelet activating factor). A factor released by basophils which causes platelets to aggregate.

PALS (periarteriolar lymphatic sheath). The accumulations of lymphoid tissue constituting the white pulp of the spleen.

Paracrine. The action of a cytokine on a cell distinct from that which produced it.

Passenger cells. Donor leucocytes present in a tissue graft that may sensitize the recipient to the graft.

Patch test. Application of antigen to skin on a patch to test for Type IV hypersensitivity reactions.

Pathogen. An organism which causes disease.

PC (phosphorylcholine). A commonly used hapten which is also found on the surface of a number of microorganisms.

PCA (passive cutaneous anaphylaxis). The technique used to detect antigen-specific IgE in which the test animal is injected intravenously with the antigen and dye, the skin having previously been sensitized with antibody.

Perforin. A granule-associated molecule of cytotoxic cells, homologous to complement C9. It can form pores on the membrane of a target cell.

Peyer's patches. Collections of lymphoid cells in the wall of the gut which form a secondary lymphoid tissue.

PFC (plaque-forming cell). An antibody-producing cell detected *in vitro* by its ability to lyse antigen-sensitized erythrocytes in the presence of complement.

PHA (phytohaemagglutinin). A mitogen for T cells.

Phagocytosis. The process by which cells engulf material and enclose it within a vacuole (phagosome) in the cytoplasm.

Phenotype. The expressed characteristics of an individual (*cf.* genotype).

MOLECULAR BIOLOGY OF
THE CELL

SECOND EDITION

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

Garland Publishing, Inc.
New York & London

TEXT EDITOR: Miranda Robertson

GARLAND STAFF

Managing Editor: Ruth Adams
Project Editor: Alison Walker
Production Coordinator: Perry Bessas
Designer: Janet Koenig
Copy Editors: Lynne Lackenbach and Shirley Cobert
Editorial Assistant: Mara Abens
Art Coordinator: Charlotte Staub
Indexer: Maija Hinkle

Bruce Alberts received his Ph.D. from Harvard University and is currently Chairman of the Department of Biophysics and Biochemistry at the University of California Medical School in San Francisco. *Dennis Bray* received his Ph.D. from the Massachusetts Institute of Technology and is currently a Senior Scientist in the Medical Research Council Cell Biophysics Unit at King's College London. *Julian Lewis* received his D.Phil. from Oxford University and is currently a Senior Scientist in the Imperial Cancer Research Fund Developmental Biology Unit, Dept. of Zoology, Oxford University. *Martin Raff* received his M.D. degree from McGill University and is currently a Professor in the Biology Department at University College London. *Keith Roberts* received his Ph.D. from Cambridge University and is currently Head of the Department of Cell Biology at the John Innes Institute, Norwich. *James D. Watson* received his Ph.D. from Indiana University and is currently Director of the Cold Spring Harbor Laboratory. He is the author of *Molecular Biology of the Gene* and, with Francis Crick and Maurice Wilkins, won the Nobel Prize in Medicine and Physiology in 1962.

Library of Congress Cataloging-in-Publication Data

Molecular biology of the cell / Bruce Alberts ... [et al.].—2nd ed.
p. cm.
Includes bibliographies and index.
ISBN 0-8240-3695-6.—ISBN 0-8240-3696-4 (pbk.)
1. Cytology. 2. Molecular biology. I. Alberts, Bruce.
[DNLM: 1. Cells. 2. Molecular Biology. QH 581.2 M718]
QH581.2.M64 1989
574.87—dc19
DNLM/DLC
for Library of Congress 88-38275
CIP

Published by Garland Publishing, Inc.
136 Madison Avenue, New York, NY 10016

Printed in the United States of America

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

subunit, is assembled on a temporary scaffold composed of a second protein. The second protein is absent from the final virus particle, and so the head structure cannot spontaneously reassemble once it is taken apart. Other examples are known in which proteolytic cleavage is an essential and irreversible step in the assembly process. This is the case for the coats of some bacterial viruses and even for some simple protein assemblies, including the structural protein collagen and the hormone insulin (Figure 3–48). From these relatively simple examples, it seems very likely that the assembly of a structure as complex as a mitochondrion or a cilium will involve both temporal and spatial ordering imparted by other cellular components, as well as irreversible processing steps catalyzed by degradative enzymes.

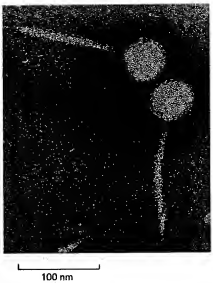

Figure 3–47 Electron micrograph of bacteriophage lambda. The tip of the virus tail attaches to a specific protein on the surface of a bacterial cell, following which the tightly packaged DNA in the head is injected through the tail into the cell. The tail has a precise length, which is determined by the mechanism shown in Figure 3–46A.

Summary

The three-dimensional conformation of a protein molecule is determined by its amino acid sequence. The folded structure is stabilized by noncovalent interactions between different parts of the polypeptide chain. The amino acids with hydrophobic side chains tend to cluster in the interior of the molecule, and local hydrogen-bond interactions between neighboring peptide bonds give rise to α helices and β sheets. Globular regions known as domains are the modular units from which many proteins are constructed; small proteins typically contain only a single domain, while large proteins contain several domains linked together by short lengths of polypeptide chain. As proteins evolved, domains were modified and combined with other domains to construct new proteins.

Proteins are brought together into larger structures by the same noncovalent forces that determine protein folding. Proteins with binding sites for their own surface can assemble into dimers, closed rings, spherical shells, or helical polymers. Although mixtures of proteins and nucleic acids can assemble spontaneously into complex structures in the test tube, many assembly processes involve irreversible steps, so that not all structures in the cell are capable of spontaneous reassembly if they are dissociated into their component parts.

Protein Function[32]

The chemical properties of a protein molecule depend almost entirely on its exposed surface residues, which are able to form weak noncovalent bonds with other molecules (see p. 88). When a protein molecule binds to another molecule, the second molecule is commonly referred to as a **ligand.** Because an effective interaction between a protein molecule and a ligand requires that many weak bonds be formed simultaneously between them, the only ligands that can bind tightly to a protein are those that fit precisely onto its surface.

The region of a protein that associates with a ligand, known as its **binding site,** usually consists of a cavity formed by a specific arrangement of amino acids on the protein surface. These amino acids often belong to widely separated regions of the polypeptide chain (Figure 3–49), and they represent only a minor fraction of the total amino acids present. The rest of the protein molecule is necessary to maintain the polypeptide chain in the correct position and to provide additional binding sites for regulatory purposes; the interior of the protein is often important only insofar as it gives the surface of the molecule the appropriate shape and rigidity.

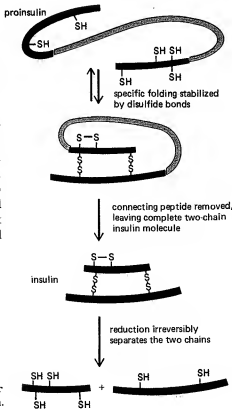

Figure 3–48 The polypeptide hormone insulin cannot spontaneously re-form if its disulfide bonds are disrupted. It is synthesized as a larger protein (*proinsulin*) that is cleaved by a proteolytic enzyme after the protein chain has folded into a specific shape. Excision of part of the proinsulin polypeptide chain causes an irretrievable loss of the information needed for the protein to fold spontaneously into its normal conformation.